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A systematic analysis of interactions between environmental risk factors and genetic variation in susceptibility to colorectal cancer

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1 **Abstract**

2 **Background:** The underlying etiology of colorectal cancer (CRC) includes both
3 genetic variation and environmental exposures. The main aim of this study was to
4 search for interaction effects between well-established environmental CRC risk factors
5 and published common genetic variants exerting main effects on CRC risk.

6 **Methods:** We used a two-phase approach: (i) Discovery phase (2,652 incident CRC
7 cases and 10,608 controls from UK Biobank) and (ii) Validation phase (1,656 cases and
8 2,497 controls from the Study of Colorectal Cancer in Scotland). Interactions with
9 nominal $P < 0.05$ in phase I were taken forward for validation in phase II. Furthermore,
10 we constructed a weighted genetic risk score (GRS) of CRC risk for each individual
11 and studied interactions between the GRS and all the environmental risk factors.

12 **Results:** Seventy of the 1,500 tested interactions were found to be nominally significant
13 in phase I. After testing these 70 interactions in phase II, the interaction between
14 rs11903757 (2q32.3/*NABPI*) and body mass index (BMI) was nominally significant
15 ($P = 0.02$) with the same direction of effects. After performing fixed-effect meta-
16 analyses to combine the results from both phases, the rs11903757*BMI interaction was
17 also found to be statistically significant (OR=1.26; 95% CI, 1.10-1.44;
18 $P_{interaction} = 6.03 \times 10^{-4}$; $P_{heterogeneity} = 0.63$). No interactions involving the GRS were
19 statistically significant in either of the two datasets.

20 **Conclusions:** Limited evidence of gene-environment interactions in CRC risk was
21 observed. There are potential modifications of the rs11903757 effect by BMI on CRC
22 risk.

23 **Impact:** Our findings might contribute to identifying subpopulations with different
24 susceptibility to the effect of BMI on CRC risk.

1 **Introduction**

2 Worldwide, colorectal cancer (CRC) is the third most common cancer by incidence and
3 second by mortality, with over 1.8 million new cases and 881,000 deaths in 2018 (1).

4 The underlying etiology of CRC includes both genetic variation and environmental
5 exposures (2). It has been suggested that the interplay between genetic variants and
6 environmental risk factors, known as gene-environment (G×E) interaction, may also
7 contribute to “missing heritability” of CRC risk (3). Thus, identification of G×E in CRC
8 risk should help to explain the undiscovered heritability of CRC, provide insights into
9 CRC etiology, and identify subpopulations with high CRC risk and potential to benefit
10 most from early intervention for CRC.

11 To date, few studies have explored G×E interactions on CRC risk (2, 4), partly because
12 assembling comprehensive datasets with both risk factor exposures and genotyping data
13 is a major challenge. We recently evaluated the evidence across the meta-analyses of
14 candidate gene studies and genome-wide G×E interaction analyses that investigated
15 G×E interactions in CRC (2). Notably, moderate strength of evidence was found for
16 some G×E interactions between several single-nucleotide polymorphisms (SNPs) and
17 alcohol drinking, processed meat intake, estrogen plus progestogen therapy use and
18 nonsteroidal anti-inflammatory drug (NSAID) use (2).

19 Two recently published genome-wide association studies (GWAS) (5, 6) have identified
20 several new genetic variants associated with CRC risk. However, the role of G×E

1 interactions involving these GWAS-identified common genetic variants underlying
2 CRC susceptibility remains largely unknown. In this study, we searched for interaction
3 effects between 100 GWAS-identified independent genetic variants (linkage
4 disequilibrium $r^2 < 0.2$) exerting main effects on CRC risk and well-established
5 environmental CRC risk factors, including standing height, body mass index (BMI),
6 smoking (status and pack-years of smoking), NSAID (aspirin and others) use, hormonal
7 replacement therapy (HRT) use, physical activity, alcohol use, and dietary intakes of
8 processed meat, red meat, vegetables, fruit, fiber and calcium. These environmental risk
9 factors were selected based on the results of meta-analyses and systematic reviews from
10 the World Cancer Research Fund International/American Institute for Cancer Research
11 Third Expert Report (7) and the subsequent Continuous Update Project Report (8). In
12 particular, it has been reported that diet low in calcium (20.5%), alcohol use (15.2%),
13 smoking (13.3%), BMI (8.6%) and diet low in fiber (11.6%) were the risk factors that
14 contributed most to disability-adjusted life-year estimates of CRC at the global level in
15 2017 (9).

16 Here, we utilize a two-phase approach to test for the interactions between common
17 genetic risk factors associated with CRC at genome-wide levels of significance and
18 environmental risk factors supported by sufficient evidence for association with CRC
19 risk from the reports (7, 8), including: (i) Discovery phase using UK Biobank data and
20 (ii) Validation phase using study samples from the Study of Colorectal Cancer in
21 Scotland (SOCCS). Furthermore, we constructed a weighted genetic risk score (GRS)
22 of CRC risk for each individual by incorporating information of the 100 independent

1 genetic variants and studied interactions between the GRS and all the environmental
2 risk factors.

3 **Materials and methods**

4 *Study population*

5 We used individual-level data from the UK Biobank cohort and SOCCS in our analysis
6 (**Table 1**).

7 **Case-control study from the UK Biobank cohort**

8 The UK Biobank is a large cohort study that has recruited more than half a million
9 people aged 40 to 69 years throughout the UK between 2006 and 2010. Questionnaire
10 data, physical measurements, blood and urine samples were collected at the baseline
11 assessment of UK Biobank (10). The web-based 24-hour dietary assessment was
12 applied to collect information on the intakes of foods and beverages consumed during
13 the 24-hour period before the assessment (11). Data abstracted from the UK Biobank
14 study consisted of 4,800 incident and prevalent CRC cases and 20,289 population-
15 based controls after the process of genotyping quality control (6). Of all the CRC cases,
16 2,652 (55.3%) were incident and 2,119 (44.1%) were prevalent cases. However, 1,907
17 (90.0%) prevalent CRC cases were diagnosed more than one year before recruitment,
18 which can be a source of bias. Therefore, we only included a total of 2,652 incident
19 CRC cases and 10,608 controls in the discovery phase of the study (**Table 1**).

1 Research ethics approval for UK Biobank to collect participant data was obtained from
2 the National Information Governance Board for Health and Social Care and the North
3 West Multicentre Research Ethics Committee. Genotypic and phenotypic data used in
4 this study were obtained from UK Biobank under an approved data request application
5 (application ID: 7441).

6 **Study of Colorectal Cancer in Scotland (SOCCS)**

7 SOCCS is a large population-based case-control study of CRC. Details of SOCCS have
8 been described previously (12). Briefly, SOCCS has recruited cases of adenocarcinoma
9 of colorectum who were aged 16-79 years in Scotland (12). Population-based controls
10 who were identified through the Community Health Index were randomly invited to
11 participate in SOCCS (12). In this study, we included a total of 1,656 CRC cases and
12 2,497 controls who had available phenotype and genetic data (**Table 1**).

13 SOCCS received research ethics approval from the MultiCentre Research Ethics
14 Committee for Scotland and relevant Local Research Ethics committees (12). All
15 participants provided written informed consent (12).

16 ***Genotyping and quality control***

17 A total of 100 GWAS-identified independent genetic variants (linkage disequilibrium
18 $r^2 < 0.2$) were examined, those identified in two recently published GWAS studies (5,
19 6). For the SNPs that were located at the same locus and in linkage disequilibrium, we
20 selected the ones that were described in Law PJ, et al. (6). For the UK Biobank genotype

1 data, biological samples of the participants were genotyped using two closely related
2 arrays from Affymetrix: the custom-designed Affymetrix UK BiLEVE Axiom array on
3 an initial 50,000 participants and Affymetrix UK Biobank Axiom array on the
4 remaining 450,000 participants. The procedure of genotyping and quality control was
5 previously reported (13). Details of phasing and imputation were previously described
6 by Bycroft et al., 2018 (13). In brief, prediction of un-genotyped variants was done
7 using IMPUTE4 software with a combination of reference panels including: (i) the
8 Haplotype Reference Consortium panel; and (ii) the merged UK10K and 1000 Genome
9 phase 3 reference panel.

10 For SOCCS, samples were genotyped using Illumina HumanHap300, HumanHap240S
11 arrays (14) and OmniExpressExome BeadChip 8v1.1, 8v1.250 or 8v1.3 (Illumina Inc.,
12 San Diego, CA) (15). Un-genotyped variants were imputed using SHAPEIT v2.837 and
13 IMPUTE v2.3.2. We used two reference panels for imputation: the 1000 Genome
14 reference panel, phase 1, December 2013 release and the UK10K reference panel
15 (release April 2014). For the X chromosome, genotypes were phased and imputed as
16 for the autosomal chromosome, with the inclusion of the “chrX” flag. X chromosome
17 variants were coded as 0 and 2 for men, assuming complete inactivation of one allele
18 in females and equal effect-size between males and females. Details of imputation and
19 subsequent quality control of imputed genotypes are given elsewhere (6).

20 ***Phenotype data***

21 Cancer cases of UK Biobank were identified through data linkage to national cancer

1 and death registries and Hospital Episode Statistics. CRC cases in UK Biobank were
2 defined using two different revisions of the International Classification of Diseases
3 (ICD), ICD-10 or ICD-9 (6). Height (standing and sitting) and weight were measured
4 during the baseline physical measurement of UK Biobank (10). Information on lifestyle
5 factors and food intakes was gathered using a self-reported touchscreen questionnaire
6 at recruitment. Information on daily intakes of nutrients was collected using a web-
7 based 24-hour dietary assessment tool about four years after the baseline assessment.
8 The 24-hour dietary assessments were performed in one-third of the UK Biobank
9 participants and was available for 947 CRC cases and 4,160 controls in our dataset. The
10 derivation of each environmental variable in the UK Biobank dataset is described in
11 **Supplementary Methods**. The environmental data were harmonized between the UK
12 Biobank dataset and the SOCCS dataset whenever possible.

13 The CRC cases of SOCCS were defined based on histologically confirmed
14 adenocarcinoma of the colon or rectum (codes 153 or 154 in ICD, 9th revision or ICD10
15 C18, C19 or C20 codes) (15). SOCCS study participants that were recruited before
16 2009 had completed two questionnaires: The Lifestyle and Cancer Questionnaire and
17 The Scottish Collaborative Group Food Frequency Questionnaire (12). The derivation
18 of each environmental variable in the SOCCS dataset has been described previously
19 (12).

20 ***Statistical methods***

21 The association between each genetic variant, each environmental risk factor and CRC

1 risk was examined by using logistic regression models. Within the UK Biobank dataset,
2 models were adjusted for age (age of CRC diagnosis for cases and age at recruitment
3 for controls), sex and assessment center, and analyses involving genetic variants were
4 further adjusted for the first 10 genetic principal components. Within the SOCCS
5 dataset, models were adjusted for age (age of CRC diagnosis for cases and age at
6 recruitment for controls) and sex. In addition, models of the analysis of dietary nutrients
7 were further adjusted for total energy intake.

8 To test for the interactions, the two-phase approach was applied. Interactions with
9 nominal P values < 0.05 in phase I were further tested in phase II. Case-control logistic
10 regression analyses including $G \times E$ interaction term(s) were applied to test for the
11 multiplicative interactions. Models were adjusted for age, sex, assessment center and
12 the first 10 genetic principal components in phase I (the UK Biobank dataset), whereas
13 models were adjusted for age and sex in phase II (the SOCCS dataset). In addition,
14 models of the interaction analysis involving dietary nutrients were further adjusted for
15 total energy intake. Furthermore, for interactions with nominal P values < 0.05 in phase
16 II, we performed fixed-effect meta-analyses to combine phase I and phase II results,
17 and obtained summary odds ratios (ORs) and 95% confidence intervals (CIs), with
18 estimation of heterogeneity measured by Cochran Q test (I^2) and its P value (16).

19 False Discovery Rate (17) was used to account for multiple testing in phase II. P values
20 unadjusted before multiple testing were termed nominal P values, whereas P values
21 after adjustment for multiple testing were termed adjusted P values and were used to

1 evaluate the statistical significance of a given interaction at the 0.05 level.

2 For interactions with nominal P values < 0.05 in phase II, we further examined (i) the
3 main effect of the environmental risk factor stratified by the SNP, (ii) the main effect of
4 the SNP stratified by the environmental risk factor and (iii) the combined association
5 stratified by both the environmental risk factor and the SNP. Also, we used an extension
6 of the Human Genome Epidemiology Network's Venice criteria (3, 18) to evaluate the
7 strength of the evidence for the $G \times E$ interactions with nominal P values < 0.05 in phase
8 II. The detailed evaluation process has been described elsewhere (2, 3).

9 To calculate the weighted GRS of CRC risk for each individual, a meta-analysis
10 excluding the UK Biobank and SOCCS study samples was first performed in order to
11 obtain unbiased regression coefficients (β -estimates) of CRC risk associated with the
12 genetic variants. Directly genotyped SNPs were coded as 0, 1 or 2 copies of the variant
13 allele. For imputed SNPs, we used the estimated number of copies of the count allele
14 (the 'dosage') with values between 0-2. Both genotyped and imputed SNPs were treated
15 as continuous variables (i.e. log-additive model). The weighted GRS was then
16 calculated by summing up the dosages of effect alleles weighted by their effect
17 estimates retrieved from the meta-analysis of GWAS and were Z-transformed to
18 normalize the distributions. Models were adjusted for the same covariates as the
19 examination of interactions between the individual SNPs and the environmental risk
20 factors.

21 Analyses were conducted using R 3.4.4 (<https://www.R-project.org/>). Power

1 calculations were performed using the Quanto software (19, 20). All statistical tests
2 were two-sided.

3 **Results**

4 Study characteristics are presented in **Table 1**. Briefly, 2,652 incident CRC cases and
5 10,608 controls from the UK Biobank cohort were included in phase I, whereas 1,656
6 cases and 2,497 controls from SOCCS were included in phase II. The summary
7 statistics of the environmental risk factors for these two datasets are presented in
8 **Supplementary Table S1**. The associations between the environmental risk factors, the
9 100 SNPs and CRC risk are presented in **Supplementary Tables S2 and S3**, separately.

10 After testing 1,500 G×E in phase I, a total of 70 G×E interactions showed nominal *P*
11 values < 0.05 (**Supplementary Table S4**). These interactions were further tested in
12 phase II, in which two interactions showed nominal *P* values < 0.05, including the
13 interactions between rs11903757 (2q32.3/*NABPI*) and BMI (nominal *P* = 0.02), and
14 rs2735940 (5p15.33/*TERT*) and smoking status (nominal *P* = 0.04) (**Table 2**). In
15 particular, the rs11903757*BMI interaction was found with the same direction of
16 effects. However, neither of the two interactions reached statistical significance after
17 accounting for multiple testing based on the 70 tests performed in phase II. After
18 performing fixed-effect meta-analyses for these two interactions, statistical significance
19 was observed for the interaction between rs11903757 and per 10 kg/m² increase in BMI
20 (OR = 1.26; 95% CI, 1.10-1.44; *P*_{interaction} = 6.03×10⁻⁴; *P*_{heterogeneity} = 0.63) (**Table 2**).
21 Furthermore, the rs11903757*BMI interaction was observed with statistical

1 significance in men after performing meta-analyses in stratified subgroups according
2 to sex (OR = 1.32; 95% CI, 1.08-1.60; $P_{interaction} = 5.53 \times 10^{-3}$; $P_{heterogeneity} = 0.78$) (**Table**
3 **2**).

4 Stratification analyses were performed for the rs11903757*BMI interaction and the
5 rs2735940*smoking status interaction in the UK Biobank dataset and the SOCCS
6 dataset, respectively (**Supplementary Tables S5 to S8**). For the rs11903757*BMI
7 interaction, above median BMI significantly increased CRC risk in individuals with TC
8 genotype (OR = 1.27; 95% CI, 1.07-1.50; $P = 5.69 \times 10^{-3}$) and non-significantly in
9 individuals with CC genotype (OR = 1.32; 95% CI, 0.70-2.52; $P = 0.393$) but not in
10 those with TT genotype ($P = 0.352$) in the UK Biobank dataset when stratified by
11 genotypes of rs11903757 (**Supplementary Table S5**). Also, the effect of BMI on CRC
12 risk stratified by genotypes of rs11903757 was limited to men in the UK Biobank
13 dataset (**Supplementary Table S5**). For the rs2735940*smoking status interaction,
14 ever smokers (compared to non-smokers) significantly increased CRC risk in
15 individuals with AA genotype (OR = 1.32; 95% CI, 1.10-1.57; $P = 2.77 \times 10^{-3}$) but not
16 in those with AG genotype ($P = 0.060$) or GG genotype ($P = 0.972$) in the UK Biobank
17 dataset when stratified by genotypes of rs2735940 (**Supplementary Table S7**).

18 **Table 3** presents the evaluation of evidence for the rs11903757*BMI interaction and
19 the rs2735940*smoking status interaction by using an extension of the Venice criteria
20 (3, 18). The environmental effects of BMI on CRC risk was graded as class III
21 (suggestive) (2) (**Supplementary Table S9**). The main effect of rs11903757

1 (2q32.3/*NABPI*) on CRC risk was graded as strong (AAA, based on the Venice criteria
 2 (18, 21)) in a meta-analysis of 12,696 cases and 15,113 controls of European descent
 3 (OR = 1.16; 95% CI, 1.10-1.22; $P = 3.71 \times 10^{-8}$; $P_{heterogeneity} = 0.27$) (**Supplementary**
 4 **Table S10**). Consequently, the interaction between rs11903757 (2q32.3/*NABPI*) and
 5 BMI was given a moderate prior score (Moderate-2) and a weak overall credibility
 6 score (**Table 3**). No evidence was found for the interaction between rs2735940
 7 (5p15.33/*TERT*) and smoking (**Table 3**).

8 **Table 4** presents the interaction effects between the weighted GRS and the
 9 environmental risk factors on CRC risk in the UK Biobank dataset and the SOCCS
 10 dataset. The distributions of the weighted GRS among the participants in the two
 11 datasets are shown in **Supplementary Figure 1 (A) and (B)**, respectively. The OR of
 12 the GRS was 1.64 (95% CI, 1.57-1.72; $P < 2 \times 10^{-16}$) in the UK Biobank dataset and 1.64
 13 (95% CI, 1.52-1.78; $P < 2 \times 10^{-16}$) in the SOCCS dataset, separately. No interactions
 14 involving the GRS were statistically significant in either of the two datasets (**Table 4**).

15 The power to detect a G×E interaction was estimated for the phase I data at a 0.05
 16 significance level, assuming a main effect of 1.10 for log-additive SNPs
 17 (**Supplementary Figures S2 to S4**). We only calculated the power for binary
 18 environmental variables because statistical power would be higher for continuous
 19 exposure variables (10). The prevalence of binary environmental exposures and the
 20 environmental ORs were chosen according to the dataset used in phase I
 21 (**Supplementary Tables S1 and S2**). With a sample size of 2,652 cases and 10,608

controls in the whole dataset, we had sufficient power (80%) to detect moderate ($OR > 1.30$) and strong ($OR > 2.00$) G×E interaction effects if the SNP was at least moderately polymorphic [minor allele frequency (MAF) = 0.20]. Similarly, the analysis of HRT use was restricted to women (1,098 cases and 4,608 controls) and the analysis of dietary nutrients was limited to the participants who had taken part in the web-based 24-hour dietary assessment of UK Biobank (947 cases and 4,160 controls in our study), we therefore had sufficient power (80%) to detect strong ($OR > 2.00$) G×E interaction effects for a moderately polymorphic (MAF = 0.20).

Discussion

Using a two-phase approach, followed by a fixed-effect meta-analysis, we searched for G×E interaction effects between 100 published common genetic variants and 15 environmental variables. Two of the 70 G×E interactions with nominal significance in phase I showed nominal P values < 0.05 in phase II, including the interactions between rs11903757 (2q32.3/*NABPI*) and BMI ($P = 0.02$), and rs2735940 (5p15.33/*TERT*) and smoking status ($P = 0.04$). In particular, the rs11903757*BMI interaction was found with the same direction of effects and showed statistical significance in the meta-analysis. No statistically significant interactions were found between the weighted GRS for CRC and the environmental risk factors in either of the two datasets.

The interaction between rs11903757 (2q32.3/*NABPI*) and BMI was nominally significant with the same direction of effects in our study. The individual effects of rs11903757 (2q32.3/*NABPI*) and BMI on CRC risk have been previously explored, but

1 the biological mechanisms behind this interaction remains unclear. Rs11903757 is an
2 intergenic SNP at 2q32.3 with closest proximity to the gene *nucleic acid binding*
3 *protein 1 (NABP1)* (44 kb centromeric) and the gene *serum deprivation response*
4 *(SDPR)* (112 kb telomeric), which encodes the serum-deprivation response
5 phosphatidylserine-binding protein (22). Also, rs11903757 is expression quantitative
6 trait loci for *NABP1* expression in whole blood ($P = 2.1 \times 10^{-15}$) and non-sun exposed
7 skin ($P = 3.2 \times 10^{-6}$) based on the results from the Genotype-Tissue Expression (GTEx)
8 project (23). Previously, the SNP rs11903757 was found to be associated with CRC risk
9 in a GWAS of European and Asian case-control studies (OR = 1.15 per risk allele; $P =$
10 3.7×10^{-8}) (22). Additionally, no statistically significant associations were observed
11 between this genotype and CRC survival in a population-based study of 5,675 patients
12 after CRC diagnosis in Scotland (24). The *NABP1* gene binds single-stranded DNA via
13 the oligonucleotide/oligosaccharide binding fold domain (25). Single-stranded DNA
14 binding proteins are essential for diverse DNA processes (22). Evidence from previous
15 biologic data also suggests that *NABP1* plays a critical role in genomic stability, which
16 could explain the development of cancer (26). BMI was used as a proxy variable of
17 body fatness in our analysis because it has been reported to be strongly correlated with
18 percentage body fat according to results from laboratory methods (10). Greater body
19 fatness, which can be measured by BMI, waist circumference and waist-to-hip ratio,
20 has been reported as a risk factor for CRC (8).

21 Hutter et al., 2012 (27) and Kantor et al., 2014 (28) performed two meta-analyses of
22 G×E interactions between a total of 26 GWAS-identified CRC risk loci and a number

1 of environmental factors. Only the interaction between rs16892766 (8q23.3/*EIF3H*)
2 and vegetable consumption showed statistical significance after accounting for
3 multiple testing in the meta-analysis with a sample size of 7,016 CRC cases and 9,723
4 controls (OR = 1.88; 95% CI, 1.36-2.59; nominal $P_{interaction} = 1.34 \times 10^{-4}$; adjusted
5 $P_{interaction} = 0.02$; $P_{heterogeneity} = 0.68$) (27). However, this interaction was not replicated
6 in phase I of our study (OR = 1.00; 95% CI, 0.85-1.17; $P = 0.996$). Additionally, the
7 rs11903757*BMI (per 10 kg/m²) interaction was found with nominal statistical
8 significance in phase II of our study and with statistical significance in the meta-
9 analysis, was not detected by Kantor, et al. (per 10 kg/m² increase; OR = 1.07; 95% CI,
10 0.94-1.22; $P_{interaction} = 0.28$; $P_{heterogeneity} = 0.45$) (28). There may be multiple reasons
11 behind these observations. One of the possible reasons is that Hutter et al. (27) and
12 Kantor et al. (28) included both nested case-control studies and case-control studies in
13 their meta-analysis, while we only used a prospective study (including 2,652 incident
14 CRC cases and 10,608 controls) in phase I and therefore will be less affected by recall
15 bias or reverse causality, when weight was already affected by the presence of cancer
16 disease.

17 The strengths of this study are that first, we examined for the first time the presence of
18 potential effect-modifications for the SNPs newly identified from the two recently
19 published meta-analyses of GWAS (5, 6). Second, we evaluated G×E interactions for a
20 wide range of environmental CRC risk factors, for which valid information was
21 collected across the studies. For the dataset used in phase I, we only used incident CRC
22 cases and controls from the UK Biobank cohort. Therefore, the information on lifestyle

1 factors and dietary habits was collected before cancer diagnosis, which minimized
2 recall bias and differential misclassifications. Though SOCCS is a case-control study,
3 participants were asked to provide information about general lifestyle and the
4 consumption of each food item one year prior to diagnosis for cases and one year prior
5 to recruitment for controls (12). Third, we critically evaluated the cumulative evidence
6 for the identified interactions using predetermined guidelines (3, 18), which have been
7 used to assess the cumulative evidence of G×E interaction effects on cancer risk (2, 4,
8 29). Lastly, for the first time we examined the interaction effects between the weighted
9 GRS for CRC and a wide variety of environmental CRC risk factors. One study has
10 examined joint effects between GRSs and plasma 25-hydroxyvitamin D (25(OH)D) on
11 CRC risk (30). However, no evidence for the modification of genetic susceptibility for
12 CRC according to vitamin D status was observed (30).

13 However, there are several limitations. First, our study had limited power to detect weak
14 ($OR < 1.30$) or moderate ($1.30 < OR < 2.00$) interactions for SNPs with MAF less than
15 0.20 even if we used the whole dataset in phase I. Furthermore, the analysis of HRT use
16 was restricted to women [hence has a reduced sample size and power] and information
17 on dietary nutrient intakes in the UK Biobank cohort was collected from the web-based
18 24-hour dietary recall assessments [found in only one-third of all UK Biobank
19 participant which again restricted sample size and power]. Therefore, further studies
20 with larger sample sizes are needed to examine the interaction effects. Second, we used
21 a prospective cohort study in phase I and a case-control study in phase II. Both types of
22 these studies have different sources of error. For case-control studies, recall bias and

1 differential misclassifications can bias estimates and may lead to false negatives. For
2 our study, cases may recall their exposure better than controls because information on
3 general lifestyle and food intakes was collected using self-reported questionnaires in
4 SOCCS. Prospective studies can minimize differential misclassification because the
5 information of lifestyle factors and dietary habits was collected for all participants at
6 recruitment. However, they may have variable time period between baseline data
7 collection and cancer diagnosis (28). In addition, we attempted to harmonize the
8 environmental variables in the UK Biobank cohort and the SOCCS, though the two
9 studies used different methods for data collection. Despite these concerns, the
10 associations between the environmental risk factors and CRC risk in the prospective
11 dataset of UK Biobank were consistent with previous observations (7, 8). Third, there
12 is a “healthy volunteer” selection bias in UK Biobank, which means that the participants
13 of UK Biobank are probably more aware of health issues than non-participants (31).
14 Therefore, the UK Biobank cohort is not fully representative of the UK general
15 population (31).

16 **Conclusion**

17 In conclusion, using a two-phase approach, we were able to observe a statistically
18 significant G×E interaction between rs11903757 (2q32.3/*NABPI*) and BMI in CRC
19 risk. Functional studies and further replications are needed to confirm our findings and
20 uncover the mechanisms of the interactions between BMI and genetic variants. Also,
21 larger studies incorporating information from consortia are needed to fully examine the

1 impact of genetic variation on the effect of BMI on CRC risk, thus to provide insights
2 into CRC etiology, and identify subpopulations who will benefit most from early
3 intervention for CRC.

4

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2 **Authors' contributions**

3 Study design: MT and ET.

4 Study concept: SMF, MGD and HC.

5 Data analysis: TY, XL and MT.

6 Manuscript draft and revision: TY, MT, ET, XL, SMF, MGD and HC.

7 Article guarantor: Dr. Maria Timofeeva and Prof. Evropi Theodoratou.

8 **Ethical approval and consent to participate**

9 Ethics approval for UK Biobank to collect participant data was obtained from the
10 National Information Governance Board for Health and Social Care and the North West
11 Multicentre Research Ethics Committee. Genotypic and phenotypic data used in this
12 study were obtained from UK Biobank under an approved data request application
13 (application ID: 7441). SOCCS received research ethics approval from the MultiCentre
14 Research Ethics Committee for Scotland and relevant Local Research Ethics
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